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(71) Applicant: DUKE UNIVERSITY [US/US]; 001E Allen Building, P.O. Box 90083, Durham, NC 27708-0083 (US).

(72) Inventors: SALING, Patricia, M.; 6 Briar Bridge Lane, Chapel Hill, NC 27516 (US). BURKS, Deborah, J.; 1020 Hale Street, Durham, NC 27705 (US). LEYTON, Lisette; Chemin de la Cavenettaz 47, CH-1053 Cugy (CH). DOWDS, Carl, A.; 4504 Linden Road, Durham, NC 27705 (US).

(74) Agents: KAGAN, Sarah, A. et al.; Banner & Allegretti, Ltd., 11th floor, 1001 G Street, N.W., Washington, DC 20001 (US).

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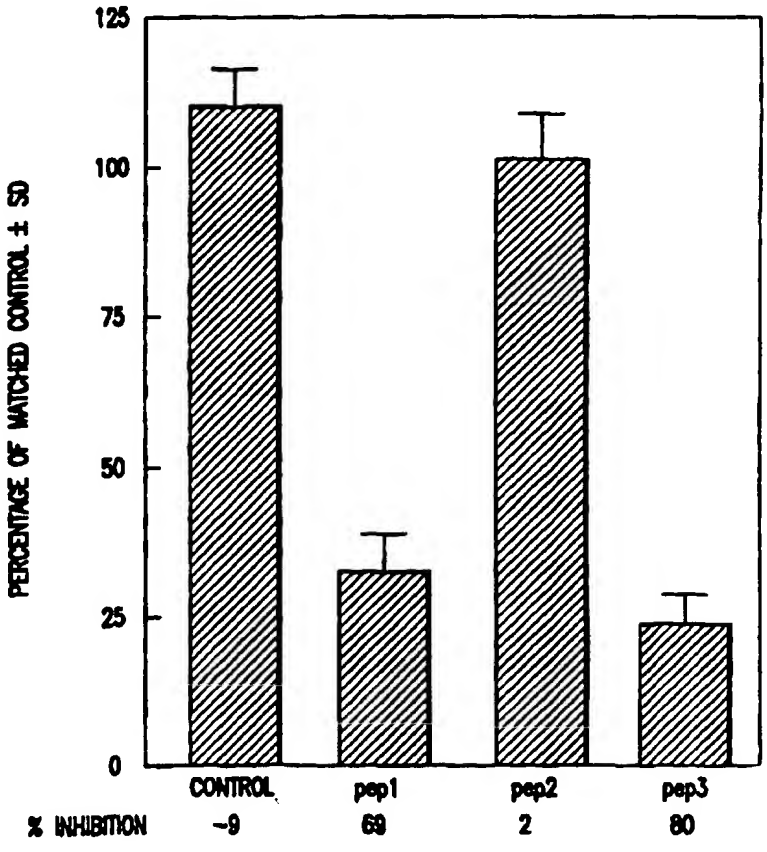
(54) Title: USE OF ZRK PEPTIDE SEQUENCES IN CONTRACEPTION

(57) Abstract

Peptide sequences that correspond to the amino terminal domain of a sperm-specific tyrosine kinase are disclosed. The peptides are able to block the binding of human sperm to human zona pellucida, the egg-specific extracellular matrix. Thus, the peptides block the interaction between sperm and egg. The peptides are used in contraceptive strategies.



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USE OF ZRK PEPTIDE SEQUENCES IN CONTRACEPTION

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BACKGROUND OF THE INVENTION

World overpopulation is a global problem that urgently needs to be alleviated and most of the measures utilized presently for birth control rely on hormonal regulation or mechanical barriers. Despite the availability of these methods, it is estimated that 50% of pregnancies in the U.S. alone are unplanned.

Novel contraceptive strategies that are targeted to the sperm or eggs specifically, in the absence of any endocrine effect, could provide an effective means of fertility control, and may provide a variety of practical advantages. An initial stumbling block to this approach is the identification of the appropriate gamete components to target. Ideal gamete components are those found only in sperm and/or eggs, those which are accessible to manipulation, and those which are essential to the fertilizing function of the gamete.

There is a need in the art for additional contraceptive methods which rely neither on hormonal regulation nor mechanical barriers.

SUMMARY OF THE INVENTION

It is an object of the invention to provide a method of inhibiting fertilization of human eggs.

It is another object of the invention to provide a polypeptide which has a contraceptive effect.

It is still another object of the invention to provide methods of screening agents for use as contraceptives.

It is yet another object of the invention to provide methods of immunizing humans to achieve a contraceptive effect.

It is an object of the invention to provide a preparation of antibodies for passively immunizing humans to achieve a contraceptive effect.

It is another object of the invention to provide vaccines for contraception.

It is still another object of the invention to provide a medicament for contraception.

It is yet another object of the invention to provide a method of producing a vaccine for contraception.

It is a further object of the invention to provide a method of producing a medicament for contraception.

It is still anothr object of the invention to provide a method for diagnosis of female infertility.

It is an object of the invention to provide a test kit for determining the presence in a body sample of antibodies to human ZRK.

It is another object of the invention to provide methods for diagnosing male infertility.

These and other objects of the invention are provided by one or more of the embodiments described below. In one embodiment a method of inhibiting fertilization of human eggs is provided. The method comprises the step of:

contacting human eggs with a polypeptide, said polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs.

In another embodiment of the invention a polypeptide is provided. The polypeptide consists of between eight and one hundred sixty-two consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, and wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs.

In yet another embodiment of the invention a method is provided for screening agents for those which are candidate agents for inhibition of human egg fertilization. The method comprises the steps of:

contacting a first substance with a second substance in the presence and absence of an agent to be tested; wherein the first substance is selected from the group consisting of: (a) human eggs, (b) isolated zona pellucida, and (c) ZP3, and the second substance is selected from the group consisting of: (a) a protein having an amino acid sequence as shown in SEQ ID NO:2, (b) a polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs, and (c) a fusion protein comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said fusion protein inhibits the binding of a human protein of SEQ ID NO:2 to human eggs;

determining the amount of the second substance bound to the first substance in the presence and absence of the agent to be tested, an agent which inhibits the binding of the second substance to said first substance being a candidate agent for inhibition of human egg fertilization.

According to another embodiment of the invention, a method is provided for testing agents for those which are candidate agents for inhibition of human egg fertilization. The method comprises the steps of:

contacting an agent to be tested with a polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs;

determining whether the agent to be tested binds to said polypeptide, an agent which binds to said polypeptide being a candidate agent for inhibition of human egg fertilization.

In yet another embodiment of the invention a method of immunizing a human to achieve a contraceptive effect is provided. The method comprises the step of:

administering to a human a polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2 in an amount effective to induce antibodies which bind a 95 kD human sperm phosphoprotein which comprises the amino acid sequence of SEQ ID NO:2.

In still another embodiment of the invention a method of passively immunizing a human to achieve a contraceptive effect is provided. The method comprises the step of:

administering to a human an antibody which specifically binds to an epitope formed by amino acids 1-161 of a 95 kD human sperm protein kinase, the amino acid sequence of said protein kinase being shown in SEQ ID NO:2.

According to another embodiment of the invention a preparation of antibodies is provided. The antibodies specifically bind to an epitope formed by amino acids 1-161 of a 95 kD human sperm protein kinase, the amino acid sequence of said protein kinase being shown in SEQ ID NO:2, said antibodies not binding to any other human sperm protein.

According to yet another embodiment of the invention a vaccine preparation is provided. The vaccine comprises: a polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs, and a pharmaceutically acceptable carrier or diluent for parenteral administration.

In an embodiment of the invention a medicament for contraception is provided. The medicament comprises: a polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs, and a pharmaceutically acceptable carrier or diluent.

In another embodiment of the invention a method of producing a vaccine is provided. The method comprises the step of:

mixing a polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs, and a pharmaceutically acceptable carrier or diluent for parenteral administration.

In still another embodiment of the invention a method of producing a medicament for contraception is provided. The medicament comprises the step of:

mixing a polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs, and a pharmaceutically acceptable carrier or diluent.

In another embodiment of the invention a method for diagnosis of female infertility is provided. The method comprises the steps of:

contacting (a) a body sample of a woman, with (b) a polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs, and

determining the presence of antibodies in said body sample which bind to said polypeptide.

In yet another embodiment of the invention a test kit for determining the presence in a body sample of antibodies to human ZRK is provided. The test kit comprises:

a polypeptide bound to a solid support, wherein said polypeptide comprises at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs.

In another embodiment of the invention a method for diagnosing male infertility is provided. The method comprises the steps of:

comparing ZRK genes of a man being tested to wild-type ZRK, a mutation in at least one of said genes of said male indicating infertility due to the inability of ZP3 to bind to sperm of said male.

In another embodiment of the invention a different method for diagnosing male infertility is provided. The method comprises the steps of:

contacting sperm of a male being tested with an antibody which specifically binds to an epitope formed by amino acids 1-161 of a 95 kD human sperm protein kinase, the amino acid sequence of said protein kinase being shown in SEQ ID NO:2; and

determining binding of said antibody to the sperm of the male being tested, failure of the sperm to bind to said antibody indicating male infertility.

Thus the present invention provides the art with a non-hormonal, non-mechanical-barrier means of contraception which prevents an early step in the conception process, preventing the formation of a zygote.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Human sperm contain a 95 kD protein that becomes tyrosine-phosphorylated during capacitation and is recognized by the mAb 97.25. Human sperm were obtained from fresh ejaculates that were allowed to liquify at RT for 1 hr and collected by centrifugation (800 xg) through a 47%/90% Percoll gradient.[25] The resulting pellet was washed twice by centrifugation (400 xg, 10 min) in TN (20 mM Tris, 130 mM NaCl, pH 7.4) buffer. Capacitation was achieved by incubating sperm in Ham's F12 medium, supplemented with 20 mM $NaHCO_3$ and 7.5% serum, at a concentration of 5 x 10^6 cells/ml for 6 hrs at 37°C in 5% CO_2.

Fig. 1a: Non-capacitated (lane 1) or capacitated (lane 2) sperm were solubilized in SDS sample buffer, the proteins separated under reducing conditions on 8% polyacrylamide gels[26] and transferred[27] to nitrocellulose membranes (Schleicher and Schuell, Keene, NH). Non-specific reactivity was blocked by incubation with 3% gelatin (cold water fish skin) in 100 mM NaCl, 50 mM Tris (pH 7.6), 0.05%

Tween-20, 0.1% NaN_3 for 1 hr. The blots were then incubated for 1 hr at RT with anti-phosphotyrosine antibody PY20 (ICN, Irvine, CA) (1 μg/ml). PY20 reactivity was visualized autoradiographically using peroxidase-conjugated goat anti-mouse IgG (KPL, Rockville, MD) followed by enhanced chemiluminescence detected (ECL Western Blot Kit, Amersham International). The reactive bands appear specific for PY since use of PY20 that was preincubated with 40 mM o-phospho-DL-tyrosine eliminated immunoreactivity with the same sperm samples (data not shown). Each lane contains 2 x 10^6 sperm. Pre-stained molecular weight markers (x 10^{-3}; BRL) were used to estimate relative molecular mass, and are indicated to the left of lane A.

Fig. 1b: Proteins from capacitated human sperm were extracted (10^6 cells/μl) in RIPA buffer (50 mM Tris HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 1% deoxycholate, 0.1% SDS)[28] in the presence of 500 KIU/ml aprotinin, 1 mM PMSF, and 500 μM Na_3VO_4. Extracted proteins were separated from insoluble material by centrifugation and then incubated overnight at 4°C with PY20 conjugated directly to Sepharose beads (10 mg IgG/ml beads; ICN). Sperm proteins extracted from 5 x 10^7 cells, representing approximately 50 μg protein, were incubated with 10 μl of packed beads. Immune complexes were washed (x3) in RIPA buffer, solubilized in SDS sample buffer, separated by SDS-PAGE, and transferred to nitrocellulose. Blots were blocked for non-specific reactivity as in Panel *a*, and then incubated overnight at 4°C with tissue culture supernatant containing mAb 97.25. Reactivity was visualized by autoradiography using ^{125}I-rabbit anti-mouse IgG + IgM (Jackson Labs, West Grove, PA; 10^6 cpm/ml). MAb 97.25 recognizes a 95 kd protein precipitated by PY20; since an indirect method of detection was used here, reaction of the second antibody with the precipitating antibody heavy chain (M_r ~ 50 kD) can also be observed.

Figure 2(A) Deduced amino acid sequence for *hu9*. An unamplified λgt11 human testis library (Clontech, Palo Alto, CA) was screened separately with PY20 and with mAb 97.25 as described.[29-31] The library was plated to achieve a plaque density of ~50,000 pfu/150 mm dish. Following IPTG induction, plaques were

transferred to nitrocellulose filters (Schleicher & Schuel) and incubated with antibody; both PY20 and mAb 97.25 were used at ~1μg/ml, the former as purified IgG and the latter as tissue culture supernatant. Positive clones were detected using an ^{125}I-labeled second antibody. 3 clones were recognized by both antibodies, and selected for restriction analysis and DNA sequencing. The isolated cDNA inserts were subcloned into pBluescript (Stratagene, La Jolla, CA) and plasmid DNA was prepared by alkaline lysis.[32] Of the positive clones, *hu9* appeared to be full-length and was selected for further analysis. Double-stranded sequencing was performed by the dideoxy method using Sequenase (USB, Cleveland, OH) and gel reagents from National Diagnostics. Sequencing primers were synthesized by Oligos Etc. and the Pathology Laboratory at University of North Carolina, Chapel Hill. DNA and amino acid sequences were analyzed using the GCG package including FASTA.[33] The nucleotide sequence has been deposited in Genbank (accession umber L08961). Predicted sequences are indicated as follows: ***bold type***, signal sequence;[34] ***underline***, potential glycosylation sites; ***double underline***, putative transmembrane domain;[18] ***shading***, invariant (or highly conserved) PTK sequences;[13] and ***underlined Y***, potential autophosphorylation sites.[13]

Fig. 2(B) Predicted structure of *Hu9*. SP, signal peptide; EC, extracellular domain; TM, transmembrane region; ▲, potential autophosphorylation sites. Numbering indicates amino acid positions.

Fig. 2(C) Comparison of *Hu9* catalytic domain with that of c-Eyk. Sequence comparison was performed using FASTA.[33] Identities are denoted by shading.

Fig. 3(a) Tissue distribution of *hu9* expression. Total RNA was isolated from the human tissues indicated using the guanidium monothiocyanate/LiCl method.[35] Ten μg of each sample were subjected to electrophoresis in 1% agarose containing formaldehyde, transferred to Nytran membranes, and probed with radiolabeled *hu9* cDNA.[32] The gel was stained with ethidium bromide to ascertain equal loading and transfer of the RNA samples. Hybridization was in 6X SSC, 50% formamide, 50 μg/ml denatured salmon sperm DNA, and 0.1X Denhardt's

at 48°C. Final washing of the blots was in 2x SSC, 0.1% SDS at 55°C. The size of the marker RNA (BM) is indicated on the left of the blot. T, testis; L, lung; Li, liver; K, kidney; S, spleen; B, brain; and O, ovary.

Fig. 3(b), Fig. 3(c) Cellular localization of *hu9* mRNA by *in situ* hybridization. Frozen tissue sections were prepared using testis collected from mature mice. Following fixation in 4% paraformaldehyde and delipidation, tissue sections were treated with acetic anhydride. Slides were prehybridized for 1 hr at 55°C. ^{35}S-riboprobes were transcribed from *hu9* plasmid DNA using T3 (sense strand) and T7 polymerase (antisense strand). Probes were subsequently hydrolyzed to yield a final probe length of 200 base pairs. Slides were hybridized with either the sense or antisense probe overnight at 55°C in 50% formamide, 10% dextran sulfate, 4X SSC, 1X Denhardt's, 0.5 mg/ml salmon sperm DNA and 10 mM DTT. Following treatment with RNAse A, slides were subjected to a stringent washing procedure including a final 30 min wash in 0.1X SSC, 10 mM EDTA at 65°C. After dehydration, slides were dipped in NTB2 emulsion, exposed to film for 4 weeks, and photographed using dark-field optics. The testis section in Fig. 3b was hybridized with the antisense probe. *Hu9* RNA transcripts detected with the antisense probe are exclusively localized within the seminiferous tubules. The density and cellular distribution of the grains observed with the antisense probe suggest postmeiotic expression of *hu9*. Control hybridization, shown in Fig. 3c, using the sense-strand *hu9* RNA, yielded an even distribution of grains that was not above background hybridization levels.

Fig. 4. An antibody generated using a synthetic peptide corresponding to residues 539-553 of the deduced sequence encoded by *hu9* recognizes a 95 kD tyrosine-phosphorylated protein in human sperm. The KLH-conjugated peptide was used to immunize a rabbit, and resulted in an ELISA titer of 1:125,000 against the peptide and 1:5,000 against KLH. Proteins from capacitated human sperm were reduced and separated on SDS gels, transferred to nitrocellulose and probed with pre-immune serum (1:200 dilution; lane A), anti-Hu9$_{539\text{-}553}$ antiserum (1:200 dilution; lane B); anti-PY recombinant RC20 (according to manufacturer;

lane C), or anti-rat brain hexokinase antiserum (1:10,000 dilution; lane D). Lanes B-D represent the same nitrocellulose sheet which has been stripped between treatments and subsequently re-probed in the order presented.

Fig. 5. Competitive hemi-zona assay identifies peptide sequences involved in human sperm-zona pellucida interaction. Excess unfertilized human eggs, which had not been exposed to sperm and would normally have been discarded, were snap frozen in Ham's F-12 medium containing 7.5-10% human serum with liquid nitrogen and maintained at $\leq$-70°C. At the time of use, vials were thawed rapidly in a 37°C bath and eggs removed directly into PBS containing 2 mM EGTA and 5% serum at 4°C. Oocytes were completely non-viable following this procedure. Zona pellucida (zp) were bisected with a micro-scalpel, and matched zp halves were used subsequently. Following bisection, zp were washed (x3) and each half-zp placed pair-wise in matched 40 μl drops of Ham's F-12 medium containing 7.5% serum. For each zp used, one half served as an internal control to which results from the other half were compared: Half A was preincubated for 30 min at 37°C with Ham's F-12 containing 7.5% serum, whereas Half B was preincubated with either Ham's F-12 containing 7.5% serum (control samples) or with peptides 1, 2, or 3 to achieve a final concentration of 10 μM. Capacitated human sperm were added to each drop (10^6 cells/ml, final conc). After 30 min, each half-zp was recovered, washed gently to remove sperm that were not tightly bound, fixed in 2% glutaraldehyde. examined with phase contrast optics and the number of sperm bound to each half-zp determined. Sperm binding levels are expressed as percentages, with the number of sperm bound to zp half B normalized to those bound to zp half A. Data were analyzed by ANOVA and paired t-test using PSPLOT software. Each treatment was evaluated using 9-10 independent hemi-zp pairs. Pep1 corresponds to residues 57-71; pep2, to residues 152-166; and pep3, to residues 94-105. All peptides were made as 1 mM stocks in Ham's F-12.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

It is a discovery of the present invention that specific sequences in the amino terminal domain of ZRK (zona receptor kinase) are important for sperm interaction with ZP3, the egg extracellular matrix protein with which a fertilizing sperm interacts initially. Sperm binding to ZP3 activates a tyrosine kinase activity intrinsic to ZRK, causing regulated exocytosis of the sperm's acrosomal granule; liberation of the enzymes sequestered therein is required for sperm penetration through the zona pellucida and access to the egg plasma membrane, where sperm-egg fusion occurs. It is a further discovery of this invention that human ZRK is a sperm protein that is fundamental to fertilization serving two essential functions in gamete interaction: it is responsible for specific recognition of the egg and it is responsible for stimulating acrosomal exocytosis in sperm. Fertilization will not occur if defects in either of these events occur.

Polypeptides derived from the sequence predicted by the *hu9* cDNA have been found to inhibit binding of ZRK to the zona pellucida, the extracellular matrix of eggs. These polypeptides are particularly useful for contraception because the polypeptide is specific to sperm, is not expressed in women, and is involved in two inter-related and essential events of the fertilization process. Moreover, the polypeptides of the invention are derived from a domain of ZRK which bears no homology with any other reported protein. The polypeptides disclosed herein, as well as other similar polypeptides which can be found using the screening method described herein, are thus suitable for use as contraceptive vaccines and contraceptive medicaments. Moreover, antibodies specific for the epitopes of ZRK corresponding to the disclosed polypeptides can be used as a means of passive immunization against sperm. In addition, DNA encoding the polypeptides of the invention can be used to produce the polypeptides, either in recombinant host cells, or in women who have been "vaccinated" with the DNA. (See Wolff et al., *Hum. Mol. Genet. 1*, 363-369, 1992, Wolff et al., *Science* 247:1465-1468, 1990, WO90/11092, WO93/17706, WO93/19183.)

According to the present invention polypeptides comprise at least eight and preferably at least ten, twelve, or fourteen consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, *i.e.*, the amino terminal domain of ZRK. The peptide inhibits the binding of a human protein of SEQ ID NO:2, *i.e.*, ZRK, to human eggs. Particularly useful segments of this sequence for forming polypeptides according to the invention are amino acids 40-151, and more particularly useful are amino acids 57-71 and amino acids 94-105. To test for other portions of the amino terminal domain which are useful for forming contraceptive polypeptides, one can test the polypeptides for the ability to inhibit ZRK binding to eggs, isolated zona pellucida, or ZP3. In one screening assay, candidate polypeptides for inhibition of human egg fertilization are identified by contacting isolated zona pellucida with ZRK, a protein having an amino acid sequence as shown in SEQ ID NO:2, in the presence and absence of the candidate polypeptide. The amount of binding of ZRK to the zona pellucida is determined. A candidate polypeptide for contraceptive activity is identified if the polypeptide significantly inhibits the binding of ZRK to human zona pellucida. Preferably the inhibition is greater than 50% of the control binding, and more preferably greater than 65% or 75%.

The polypeptide of the invention may be covalently attached to another substance, such as keyhole limpet hemocyanin (KLH) in order to stimulate a larger immune response. The polypeptide may also contain repeated units of active portions of ZRK, in order to stimulate a larger response than a single unit provides.

DNA encoding the polypeptides of the invention can be used to produce the polypeptides in recombinant host cells. Techniques for producing expression vectors and transfectants are well known in the art. Suitable host cells are also known and can be selected according to the desired purpose and cell characteristics. While any DNA which encodes the polypeptides can be used, one may desire to use the nucleotide sequence as it occurs in humans. The human sequence is shown in SEQ ID NO:1. Antisense constructs can also be used in order to inhibit the expression of ZRK in males. Production of antisense

constructs are well known in the art. It may also be desirable to use the nucleotide sequence encoding a suitable peptide according to the invention for "vaccination". According to this method, DNA encoding a particular polypeptide is administered to a recipient and the recipient expresses the protein, against which the recipient also has an immune response. Such immunizations are typically intramuscular, but may also be intraperitoneal, intravenous, or subcutaneous.

Immunization with polypeptide vaccines is also contemplated by the present invention. Such immunizations may be intramuscular, intraperitoneal, intravenous or subcutaneous. Polypeptide vaccines are typically formulated in a pharmaceutically acceptable carrier or diluent for parenteral administration.

The polypeptides of the invention may also be used as a medicament rather than as a vaccine. That is, the polypeptides can be used as a contraceptive medicament which is administered at or near the time of intercourse. The polypeptides thus function as active competitors of the fertilization reaction. Medicaments for such uses are typically formulated from a pharmaceutically acceptable carrier or diluent for vaginal delivery.

The polypeptides of the present invention can also be used diagnostically. They can be used to screen women for certain anti-sperm immune responses which cause or contribute to infertility or low fertility. In one suitable assay, the polypeptide of the invention is coated on a solid support, such as a microtiter plate. A body sample, such as serum, from the woman being tested is applied to the solid support coated with polypeptide. If there are anti-ZRK antibodies in the body sample they will bind to the polypeptide. Such antibodies can be detected, *e.g.*, using anti-human immunoglobulin antibodies which are detectably labeled.

Such assays readily lend themselves to formulation in kits which can be conveniently used by physicians or clinical laboratories. The kit may contain a solid support, such as a plate or beads, which has been pre-coated with polypeptide of the invention. Anti-human immunoglobulin antibodies, labeled or not, may also be included. Instructions for carrying out the assay and calibration curves may also be included.

Similarly, male causes of infertility may also be detected according to the findings of the present invention. Sperm can be screened using antibodies which specifically bind to the polypeptides of the invention. Sperm which fail to bind to such antibodies would indicate a mutation in or affecting ZRK. In another assay for causes of male infertility, the ZRK gene of a man can be examined to determine whether a mutation has occurred which affects ZRK expression or function. Mutations can be determined by comparison to the wild-type sequence shown in SEQ ID NO:1.

In order to screen agents for those which are candidates to inhibit human egg fertilization, one can employ the discovery of the present invention. Specifically one can contact a first substance with a second substance in the presence and absence of an agent to be tested. The first substance is selected from the group consisting of: (a) human eggs, (b) isolated zona pellucidae, and (c) ZP3, and the second substance is selected from the group consisting of: (a) a protein having an amino acid sequence as shown in SEQ ID NO:2, (b) a polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs, and (c) a fusion protein comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said fusion protein inhibits the binding of a human protein of SEQ ID NO:2 to human eggs. The amount of the second substance bound to the first substance in the presence and absence of the agent to be tested is determined. An agent which inhibits the binding of the second substance to said first substance is identified as a candidate agent for inhibition of human egg fertilization. Techniques for determining binding of one protein to another are well known in the art and any such technique as is convenient may be used.

Similarly an agent to be tested can be contacted with a polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs. The binding of the agent to be tested to said polypeptide

is then determined. An agent which binds to said polypeptide is identified as a candidate agent for inhibition of human egg fertilization.

Examples

Example 1

This example demonstrates the existence of a human sperm protein that becomes tyrosine-phosphorylated during capacitation.

Regulated exocytosis of the sperm acrosome, also called the acrosome reaction (AR), is a consequence of ZP3 binding[2] and is mandatory for fertilization. However, the molecular mechanisms whereby sperm binding to ZP3 triggers exocytosis are not well understood. Tyrosine phosphorylation in sperm appears important for fertilization, since inhibition of protein tyrosine kinase (PTK) activity prevents acrosomal exocytosis,[5] and blocks fertilization. Recently, a 95 kD mouse sperm membrane protein has been identified as a receptor for mouse ZP3.[3] Several lines of evidence indicate that this receptor is a member of the PTK receptor family: i) it contains phosphotyrosine (PY), the level of which increases upon exposure to zp proteins,[3] and ii) PTK activity is stimulated by direct exposure of the isolated receptor to ligand.[5] Further analogy with PTK receptors includes the finding that ZP3 receptor aggregation is an initiating signal in the cascade leading to acrosomal exocytosis.[6] Given these characteristics, we referred to this mouse 95 kD protein as zona receptor kinase (ZRK).

The evolutionary conservation of PTK receptors,[7-9] as well as the demonstrated similarity between mouse and human ZP3,[10] suggested the existence of a ZRK functional homolog in human sperm. The presence of PY-containing proteins in human sperm was addressed by Western analysis using the anti-PY antibody PY20.[11] In parallel with findings in mice,[3] a 95 kD protein was the major PY-containing protein identified in human sperm (Fig. 1a, lane 1), and its level of PY increases with capacitation (Fig. 1a, lane 2), a final maturational process that primes sperm for zp-triggered ARs.[12] Since a 95 kD human sperm protein, identified using mAb 97.25, had already been implicated in sperm-zona interaction,[4] we investigated whether the 97.25 antigen was tyrosine-

phosphorylated. When mAb 97.25 was used to probe blots of PY20-immunoprecipitated human sperm proteins (Fig. 1b), a 95 kD protein was detected, suggesting that the 95 kD protein in human sperm recognized by both anti-PY and mAb 97.25 is a functional homolog of mouse sperm ZRK.

Example 2

This example shows the sequence of a human tyrosine kinase cloned from testis DNA.

We utilized PY20 and mAb 97.25, targeted respectively to intracellular and extracellular domains of ZRK, as sequential probes to select a clone which expresses human ZRK. From a human testis cDNA expression library, we have isolated a clone, *hu9*, which is reactive with both antibodies. Sequencing of the 2.2-kb insert revealed an open reading frame of 1,800 nucleotides, predicting a protein of 600 amino acids with a molecular weight of ~68,000 (Fig. 2A). The *hu9* cDNA appears to be full-length based on Northern analysis (Fig. 3). Comparison of *hu9* with sequences in the Genbank and Swiss Protein data bases revealed that this cDNA encodes a novel PTK. The predicted amino acid sequence of *hu9* contains motifs found in all PTKs[13] (Fig. 2), including the highly conserved ATP-binding site (GEGEKG, residues 249-254) and highly conserved sequences from the eleven major catalytic subdomains of these molecules. Two potential autophosphorylation sites lie within 20 residues upstream of the Ala-Pro-Ile consensus triplet in subdomain VIII of Hu9. Hu9 has little homology to other PTKs in subdomains X and XI, which are the least conserved catalytic domains.[13] Subdomain X, often consisting of approximately 20 residues in other PTKs,[13] is represented by 30 residues in Hu9. The deduced amino acid sequence of the Hu9 catalytic domain is most similar (55% identity) to that of c-Eyk, a receptor-like PTK identified recently as the proto-oncogene of v-eyk (v-ryk);[14] potential ligands for this new PTK receptor subfamily[15-17] have not yet been reported. Hu9 contains other structural features common to PTK receptors (Fig. 2B), including a sequence of hydrophobic amino acids (residues 162-182) capable of serving as a transmembrane region[18] and an amino terminal extracellular domain that could

possess the ligand binding site(s). However, comparison of the Hu9 extracellular domain with other database logged sequences yielded no significant homology. The putative Hu9 extracellular domain contains several potential glycosylation sites and is cysteine-rich but the 9 Cys residues present in Hu9 are not arranged in typical clusters, as observed in other PTK receptors.[19]

Example 3

This example shows the tissue distribution and cellular localization of *hu9* expression.

When Northern blots containing RNA from various human tissues were probed with the *hu9* cDNA, a single transcript of approximately 2.2 kb was detected in human testis, suggesting the cloned insert represents the complete mRNA encoding the Hu9 protein (Fig. 3a). *Hu9* did not hybridize with RNA from the other human tissues surveyed. Furthermore, *hu9* detected a single transcript of 2.7 kb in RNA prepared from mouse testis (data not shown). These results suggest that *hu9* expression is testis-specific and that a homolog of this protein exists in mouse testis. Because mature sperm are transcriptionally inactive, it is not feasible to prepare RNA from these cells for Northern analysis. Therefore, *in situ* hybridization was used to examine the distribution of the *hu9* transcript within the testis (Fig. 3b, c); localization was confined to post-meiotic germ cells as demonstrated by the grain pattern on cross-sections through germinal epithelia, indicating that the *hu9* transcript is specific to germ cells.

Example 4

This example shows that the sperm protein encoded by *hu9* is not hexokinase.

To investigate characteristics of the protein encoded by *hu9* in mature sperm, a polyclonal antibody was prepared using a synthetic peptide that corresponds to a unique portion of the intracellular domain of the deduced polypeptide (residues 539-553). This antibody recognized a 95 kD human sperm protein that contains PY (Fig. 4, lanes B, C). Contrary to a recent suggestion[20]

however, the protein that is recognized by anti-$Hu9_{539-553}$ as well as anti-Py antibodies is distinct from hexokinase (Fig. 4, lane D).

Example 5

This example shows that selected portions of the *hu9*-encoded polypeptide are able to inhibit the binding of sperm to human zona pellucida.

The putative receptor function of the *hu9*-encoded polypeptide was addressed in a competitive sperm-zp binding assay, using a hemi-zona assay design.[21-24] Human zp were preincubated with synthetic peptides corresponding to different regions of the predicted extracellular domain and were then challenged with sperm to determine the potential of these sequences to compete with sperm for binding sites (Fig. 5). Two of the peptides tested (pep1 [residues 57-71] and pep3 [residues 94-105]) caused significant inhibition of sperm-zp interaction, blocking binding by 69% and 80%, respectively. In contrast, a third peptide (pep2 [residues 152-166]) produced no effect on binding levels.

Our data indicate that *hu9* encodes a transmembrane PTK expressed exclusively in sperm and spermatogenic cells of the testis, that antibodies directed against a unique portion of the deduced intracellular domain recognize a 95 kD tyrosine-phosphorylated human sperm protein, and that peptides corresponding to specific regions of the amino terminal domain effectively inhibit human sperm interaction with the zona pellucida.

All references cited herein are incorporated by reference.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Duke University

(ii) TITLE OF INVENTION: USE OF ZRK PEPTIDE SEQUENCES IN CONTRACEPTION

(iii) NUMBER OF SEQUENCES: 2

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Banner & Allegretti, Ltd.
(B) STREET: 1001 G Street, N.W.
(C) CITY: Washington
(D) STATE: D.C.
(E) COUNTRY: USA
(F) ZIP: 20001

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/289,119
(B) FILING DATE: 11-AUG-1994
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Kagan, Sarah A.
(B) REGISTRATION NUMBER: 32,141
(C) REFERENCE/DOCKET NUMBER: 00240.46180

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 202-508-9100
(B) TELEFAX: 202-508-9299
(C) TELEX: 97430 BBMB UT

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1875 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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TGTTTCTGAA TGAAGCCACC TATAAAGCAA CAAGGTGAGA AGGGTGGCTC AAGGATCTCC 120

CATGGTTGGC AAAGCGCTGG CATTTCTAAA GAATTATTAG AAGAAGGTGG GCAAAATGGG 180

TCTCGTGCGC GTATTTCTGT TCAAGTCCAT AACGCCACCT GCACCGTGAG GATTGCCGCT 240

GTTACCAAGG GGGGAGTCGG CCCTTTGTCT GACCCCGTGT GTAAATATTA TACAGGAGGG 300

AATACCGGGT ATTTCTGCGC TAAGGTATCC CGCATGTCCA CACATAGAAG CTTTAAACAA 360

GATGACACCT TACACATCCC CTGTCGAGGG CGACCAGAAC CAAACGTGAC CTGCCGAGAC 420

CTAAAGCGGT GCAATGTGTC CGACGAAGTT CAAAGGGGCA TGCCAGGGAA CGTCACACCC 480

TGCACACGAC TAGGCCGGCT CTGTCCGCTA TTTAACTCAG GCGCCTGGCA ACGCAGATCC 540

TGTGCTCATC ATCTTTGGCT GCTTTTGTGG ATTATTTTGA TTGGGTTGGT TTTATACATC 600

TCCTGGGCCA TCAGAAAAAG AGTCCAGGAG ACAAAGTTTG GGAATGCATT CACAGAGGAG 660

GATTCTGAAT TAGTGGTGAA TTATATAGCA AAGAAATCCT TCTGTCGGCG AGCCATTGAA 720

CTTACCTTAC ATAGCTTGGG AGTCAGTGAG GAACTACAAA ATAAACTAGA AGATGTTGTG 780

ATTGACAGGA ATCTTCTAAT TCTTGGAAAA ATTGTGGGTG AAGGAGAGAA AGGGACCGTG 840

TATGAAGGAC TGTGGAATAT CCCCGAAGGA AAGGAAGTAA AAATTCCAGT AGCAATCAAG 900

ACCCTAAAAT GGGACACTAT GGCTAATAAA GAAATACTTG ATGAAAGTGT CATGAAAGGC 960

TTTGGGAACC CCCACGTAGT GCGACTCCTT GGGATATGTA TGACATCTAC AATATATGTA 1020

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TCAAATTGTG CCGAGCTAAA CCCACCGCTG CAGACACTAT TGAAGTTCAT GGTGGATATT 1140

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AACTGCATGT TGCGAGATGA CATGACTGTC TGTGTTGCGG ACTTCGGCCT CTCTAAGAAG 1260

ATTTACAGTG GCGATTATTA CCGCCAAGGC CGCATTGCTA AGATGCCTGT TAAATGGATC 1320

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AACCATGAGA TGTACGACTA CCTTCTCCAC GGCCACAGGC TGAAGCAGCC TCGAACCGCT 1500

TGGAACTGTA CAGAAATCCG AATCCGACTC CTAAAACTAC CAATCCTGGG GAGTCGAACA 1560

ATGCGTCCAA TGACAATATT TTCAATGGCC ACGCGACTAT CATCACCCAA AACAGCTTGG 1620

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TTCTCGGTAC TAAGACTACA GCTGGAAAAA TTGCTAGAGA GCTTACCGGA CGTTAGAAAT 1740

CAAGCAGATG TAATTTATGT TAACACTCAA TTGCTAGAGA GCGAAGGCCT TGCCAGGGTT 1800

CATCCCTGTA GTACCGGGCT CGAACACCAT CCATGCAGCG AACATCGACC GCGTCCGCAC 1860

CTCTATAATT GCTAA 1875

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 600 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Lys Pro Ile Thr Lys Gln Gln Gly Glu Leu Val Gly Ser Arg Ile
1 5 10 15

Ser His Val Trp Gln Ser Ala Gly Ile Ser Lys Glu Leu Leu Glu Glu
20 25 30

Val Gly Gln Asn Gly Ser Arg Ala Arg Ile Ser Val Gln Val His Asn
35 40 45

Ala Thr Cys Thr Val Arg Ile Ala Ala Val Thr Lys Gly Gly Val Gly
50 55 60

Pro Phe Ser Asp Pro Val Cys Lys Tyr Tyr Thr Gly Gly Asn Thr Gly
65 70 75 80

Tyr Phe Cys Ala Asn Val Ser Arg Met Ser Thr His Arg Ser Phe Lys
85 90 95

Leu Asn Asn Thr Leu His Ile Pro Cys Arg Gly Arg Pro Gln Pro Asn
100 105 110

Val Thr Cys Arg Asp Leu Lys Arg Cys Asn Val Ser Asp Glu Val Gln
115 120 125

Arg Gly Met Pro Gly Asn Val Thr Pro Cys Thr Arg Leu Gly Arg Leu
130 135 140

Cys Pro Leu Phe Asn Ser Gly Ala Trp Gln Arg Arg Ser Cys Ala His
145 150 155 160

His Leu Trp Leu Leu Leu Trp Ile Ile Leu Ile Gly Leu Val Leu Tyr
165 170 175

Ile Ser Leu Ala Ile Arg Lys Arg Val Gln Glu Thr Lys Phe Gly Asn
180 185 190

Ala Phe Thr Glu Glu Asp Ser Glu Leu Val Val Asn Tyr Ile Ala Lys
195 200 205

Lys Ser Phe Cys Arg Arg Ala Ile Glu Leu Thr His Ser Leu Gly Val
210 215 220

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Ser Glu Glu Leu Gln Asn Lys Leu Glu Asp Val Val Ile Asp Arg Asn
225                 230                 235                 240

Leu Leu Ile Leu Gly Lys Ile Leu Gly Glu Gly Glu Lys Gly Thr Val
                245                 250                 255

Tyr Glu Gly Leu Trp Asn Ile Pro Glu Gly Lys Glu Val Lys Ile Pro
            260                 265                 270

Val Ala Ile Lys Thr Leu Lys Leu Asp Thr Met Ala Asn Lys Glu Ile
        275                 280                 285

Leu Asp Glu Ala Ser Val Met Lys Gly Phe Gly Asn Pro His Val Val
    290                 295                 300

Arg Leu Leu Gly Ile Cys Met Thr Ser Thr Ile Tyr Val Ile Thr Glu
305                 310                 315                 320

Tyr Cys Leu Leu Val Tyr Arg Arg Asn Lys Asp Lys Ala Glu Gln His
                325                 330                 335

Arg Ser Asn Cys Ala Glu Leu Asn Pro Pro Leu Gln Thr Leu Leu Lys
            340                 345                 350

Phe Met Val Asp Ile Ala Leu Gly Met Glu Tyr Leu Ser Asn Arg Asn
        355                 360                 365

Phe Leu His Arg Asp Leu Ala Ala Arg Asn Cys Met Leu Arg Asp Asp
    370                 375                 380

Met Thr Val Cys Val Ala Asp Phe Gly Leu Ser Lys Lys Ile Tyr Ser
385                 390                 395                 400

Gly Asp Tyr Tyr Arg Gln Gly Arg Ile Ala Lys Met Pro Val Lys Trp
                405                 410                 415

Ile Ala Ile Glu Ser Leu Ala Asp Arg Val Tyr Thr Lys Ser Asp Val
            420                 425                 430

Trp Ala Phe Gly Val Thr Met Trp Glu Ile Ala Thr Thr Leu Arg Gly
        435                 440                 445

Met Thr Pro Tyr Pro Gly Val Gln Asn His Glu Met Tyr Asp Tyr Leu
    450                 455                 460

Leu His Gly His Arg Leu Lys Gln Pro Arg Thr Ala Trp Asn Cys Thr
465                 470                 475                 480

Glu Ile Arg Ile Arg Leu Leu Lys Leu Pro Ile Leu Gly Ser Arg Thr
                485                 490                 495

Met Arg Pro Met Thr Ile Phe Ser Met Ala Thr Arg Leu Ser Ser Pro
            500                 505                 510

Lys Thr Ala Trp Met Asn Cys Met Lys Lys Cys Thr Leu Ala Gly Glu
        515                 520                 525

Pro Ile Pro Lys Thr Gly Pro Thr Phe Ser Val Leu Arg Leu Gln Leu
    530                 535                 540

Glu Lys Leu Leu Glu Ser Leu Pro Asp Val Arg Asn Gln Ala Asp Val
545                 550                 555                 560
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Ile Tyr Val Asn Thr Gln Leu Leu Glu Ser Glu Gly Leu Ala Arg Val
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His Pro Cys Ser Thr Gly Leu Glu His His Pro Cys Ser Glu His Arg
            580                 585                 590

Pro Arg Pro His Leu Tyr Asn Cys
        595                 600
```

CLAIMS

1. A method of inhibiting fertilization of human eggs, comprising the step of:

contacting human eggs with a polypeptide, said polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs.

2. A polypeptide consisting of between eight and one hundred sixty-two consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs.

3. The polypeptide of claim 2 which consists of amino acids 57-71 of SEQ ID NO:2.

4. The polypeptide of claim 2 which consists of amino acids 94-105 of SEQ ID NO:2.

5. The polypeptide of claim 2 which consists of amino acids selected from amino acids 40-151.

6. The polypeptide of claim 2 which consists of amino acids selected from amino acids 40-161.

7. An isolated DNA molecule which encodes a polypeptide according to any of claims 2 to 6.

8. The DNA molecule of claim 7 having a nucleotide sequence which consists of all or a portion of nucleotides 70 to 1875 as shown in SEQ ID NO:1, or the complement thereof.

9. A method of screening agents for those which are candidate agents for inhibition of human egg fertilization, comprising the steps of:

contacting a first substance with a second substance in the presence and absence of an agent to be tested; wherein the first substance is selected from the group consisting of: (a) human eggs, (b) isolated zona pellucidae, and (c) ZP3, and the second substance is selected from the group consisting of: (a) a

protein having an amino acid sequence as shown in SEQ ID NO:2, (b) a polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs, and (c) a fusion protein comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said fusion protein inhibits the binding of a human protein of SEQ ID NO:2 to human eggs;

determining the amount of the second substance bound to the first substance in the presence and absence of the agent to be tested, an agent which inhibits the binding of the second substance to said first substance being a candidate agent for inhibition of human egg fertilization.

10. A method of testing agents for those which are candidate agents for inhibition of human egg fertilization, comprising the steps of:

contacting an agent to be tested with a polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs;

determining whether the agent to be tested binds to said polypeptide, an agent which binds to said polypeptide being a candidate agent for inhibition of human egg fertilization.

11. A method of immunizing a human to achieve a contraceptive effect, comprising the step of:

administering to a human a polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2 in an amount effective to induce antibodies which bind a 95 kD human sperm phosphoprotein which comprises the amino acid sequence of SEQ ID NO:2.

12. The method of claim 11 wherein said polypeptide comprises amino acids 57-71 of SEQ ID NO:2.

13. The method of claim 11 wherein said polypeptide comprises amino acids 94-105 of SEQ ID NO:2.

14. The method of claim 11 wherein said polypeptide comprises amino acids selected from amino acids 40-151.

15. The method of claim 11 wherein said polypeptide comprises amino acids selected from amino acids 40-161.

16. A method of passively immunizing a human to achieve a contraceptive effect, comprising the step of:

administering to a human an antibody which specifically binds to an epitope formed by amino acids 1-161 of a 95 kD human sperm protein kinase, the amino acid sequence of said protein kinase being shown in SEQ ID NO:2.

17. A preparation of antibodies which specifically bind to an epitope formed by amino acids 1-161 of a 95 kD human sperm protein kinase, the amino acid sequence of said protein kinase being shown in SEQ ID NO:2, said antibodies not binding to any other human sperm protein.

18. A vaccine preparation comprising: a polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs, and a pharmaceutically acceptable carrier or diluent for parenteral administration.

19. A medicament for contraception comprising: a polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs, and a pharmaceutically acceptable carrier or diluent.

20. A method of producing a vaccine comprising the step of:

mixing a polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs, and a pharmaceutically acceptable carrier or diluent for parenteral administration.

21. A method of producing a medicament for contraception, comprising the step of:

mixing a polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs, and a pharmaceutically acceptable carrier or diluent.

22. A method for diagnosis of female infertility, comprising the steps of:

contacting (a) a body sample of a woman, with (b) a polypeptide comprising at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs, and

determining the presence of antibodies in said body sample which bind to said polypeptide.

23. A test kit for determining the presence in a body sample of antibodies to human ZRK, comprising:

a polypeptide bound to a solid support, wherein said polypeptide comprises at least eight consecutive amino acids of amino acids 1-161 of SEQ ID NO:2, wherein said polypeptide inhibits the binding of a human protein of SEQ ID NO:2 to human eggs.

24. The test kit of claim 23 further comprising:

antibodies immunoreactive with human immunoglobulin.

25. The test kit of claim 24 wherein said antibodies are detectably labeled.

26. A method for diagnosing male infertility, comprising the steps of:

comparing ZRK genes of a man being tested to wild-type ZRK, a mutation in at least one of said genes of said male indicating infertility due to the inability of ZP3 to bind to sperm of said male.

27. A method for diagnosing male infertility, comprising the steps of:

contacting sperm of a male being tested with an antibody which specifically binds to an epitope formed by amino acids 1-161 of a 95 kD

human sperm protein kinase, the amino acid sequence of said protein kinase being shown in SEQ ID NO:2; and

determining binding of said antibody to the sperm of the male being tested, failure of the sperm to bind to said antibody indicating male infertility.

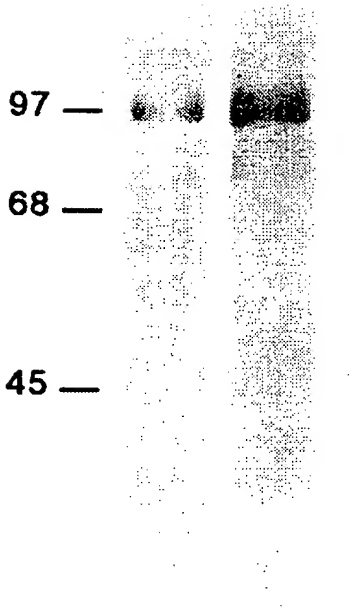

FIG.1A

FIG.1B

```
MKPITKQQGELVGSRISHVWQSAGISKELLEEVGQNGSRARISVQVHNAT   50
CTVRIAAVTKGGVGPFSDPVCKYYTGGNTGYFCANVSRMSTHRSFKLNNT  100
LHIPCRGRPQPNVTCRDLKRCNVSDEVQRGMPGNVTPCTRLGRLCPLFNS  150
GAWQRRSCAHHLWLLLWIILIGLVLYISLAIRKRVQETKFGNAFTEEDSE  200
LVVNYIAKKSFCRRAIELTHSLGVSEELQNKLEDVVIDRNLLILGKILGE  250
GEKGTVYEGLWNIPEGKEVKIPVAIKTLKLDTMANKEILDEASVMKGFGN  300
PHVVRLLGICMTSTIYVITEYCLLVYRRNKDKAEQHRSNCAELNPPLQTL  350
LKFMVDIALGMEYLSNRNFLHRDLAARNCMLRDDMTVCVADFGLSKKIYS  400
GDYYRQGRIAKMPVKWIAIESLADRVYTKSDVWAFGVTMWEIATTLRGMT  450
PYPGVQNHEMYDYLLHGHRLKQPRTAWNCTEIRIRLLKLPILGSRTMRPM  500
TIFSMATRLSSPKTAWMNCMKKCTLAGEPIPKTGPTFSVLRLQLEKLLES  550
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FIG.2A

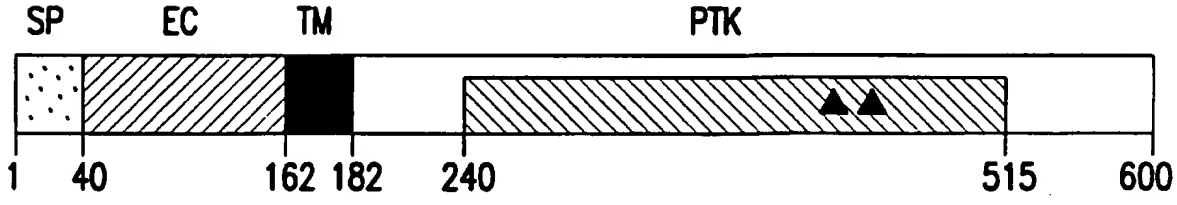
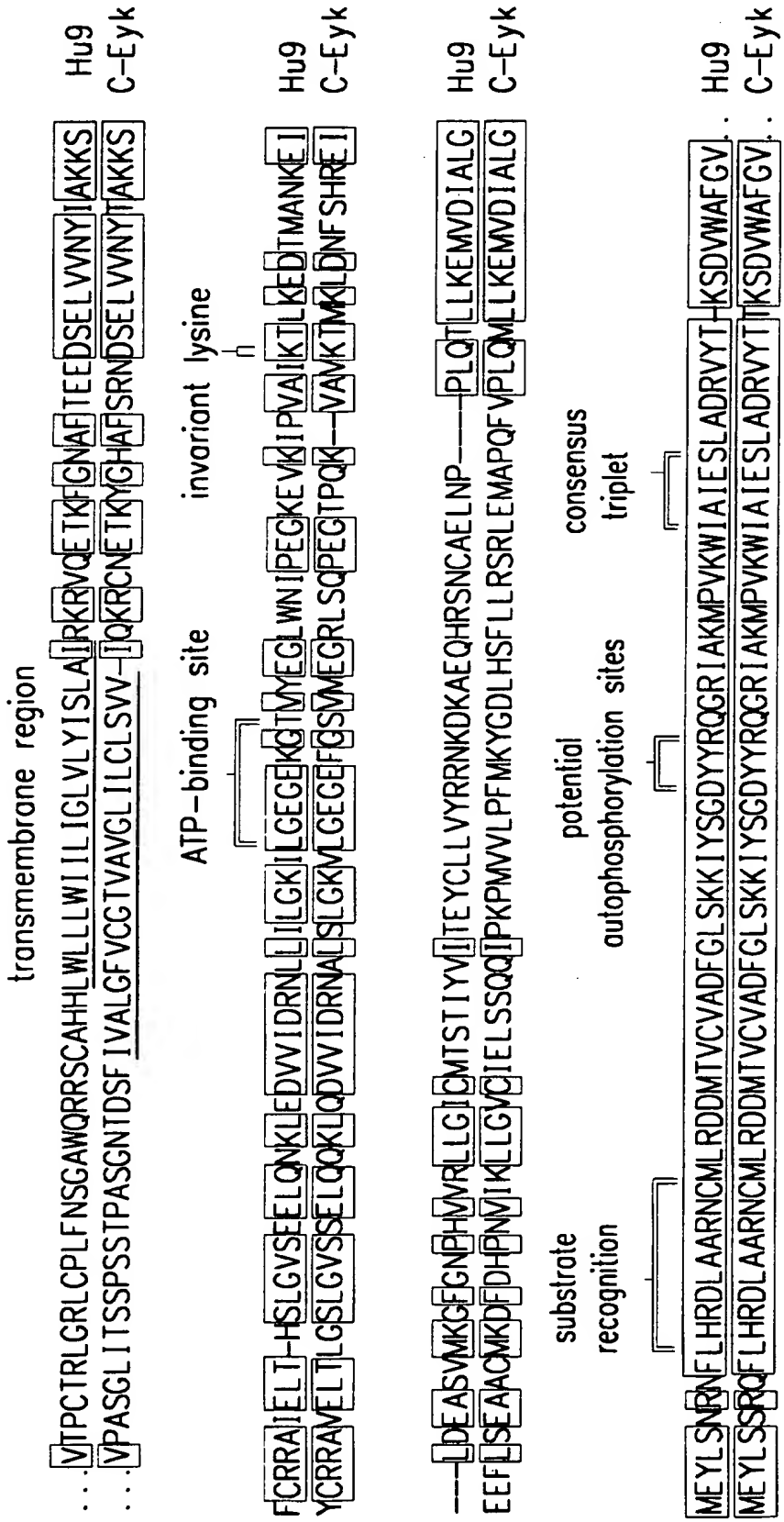


FIG.2B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ATP-binding site
invariant lysine
substrate recognition
potential autophosphorylation sites
consensus triplet
Hu9
C-Eyk

FIG.2C

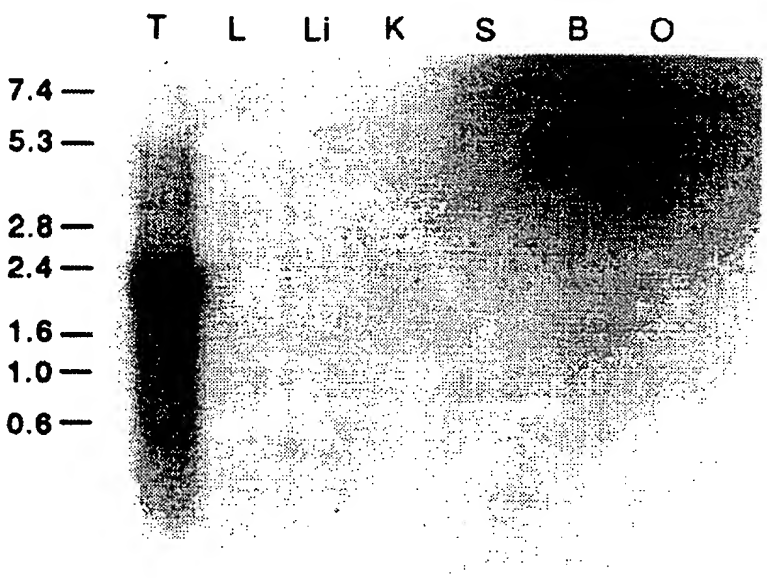

FIG.3A

FIG.3B

FIG.3C

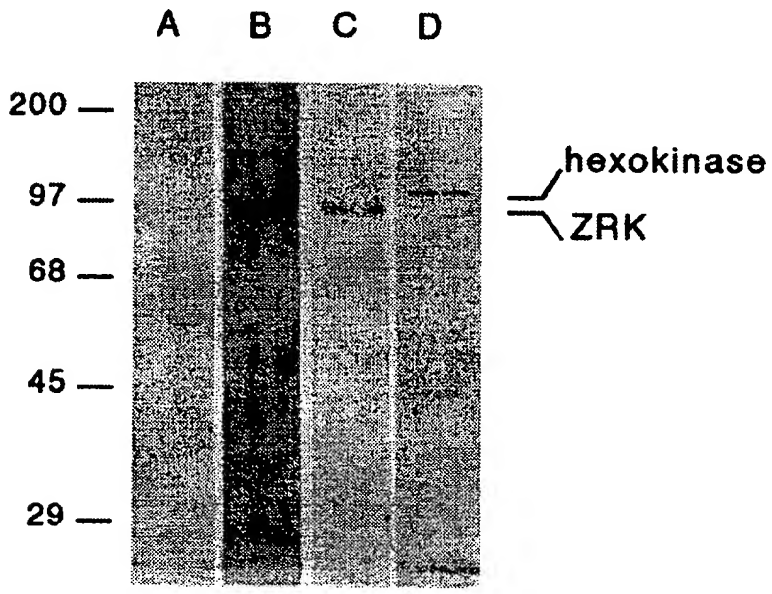

FIG.4

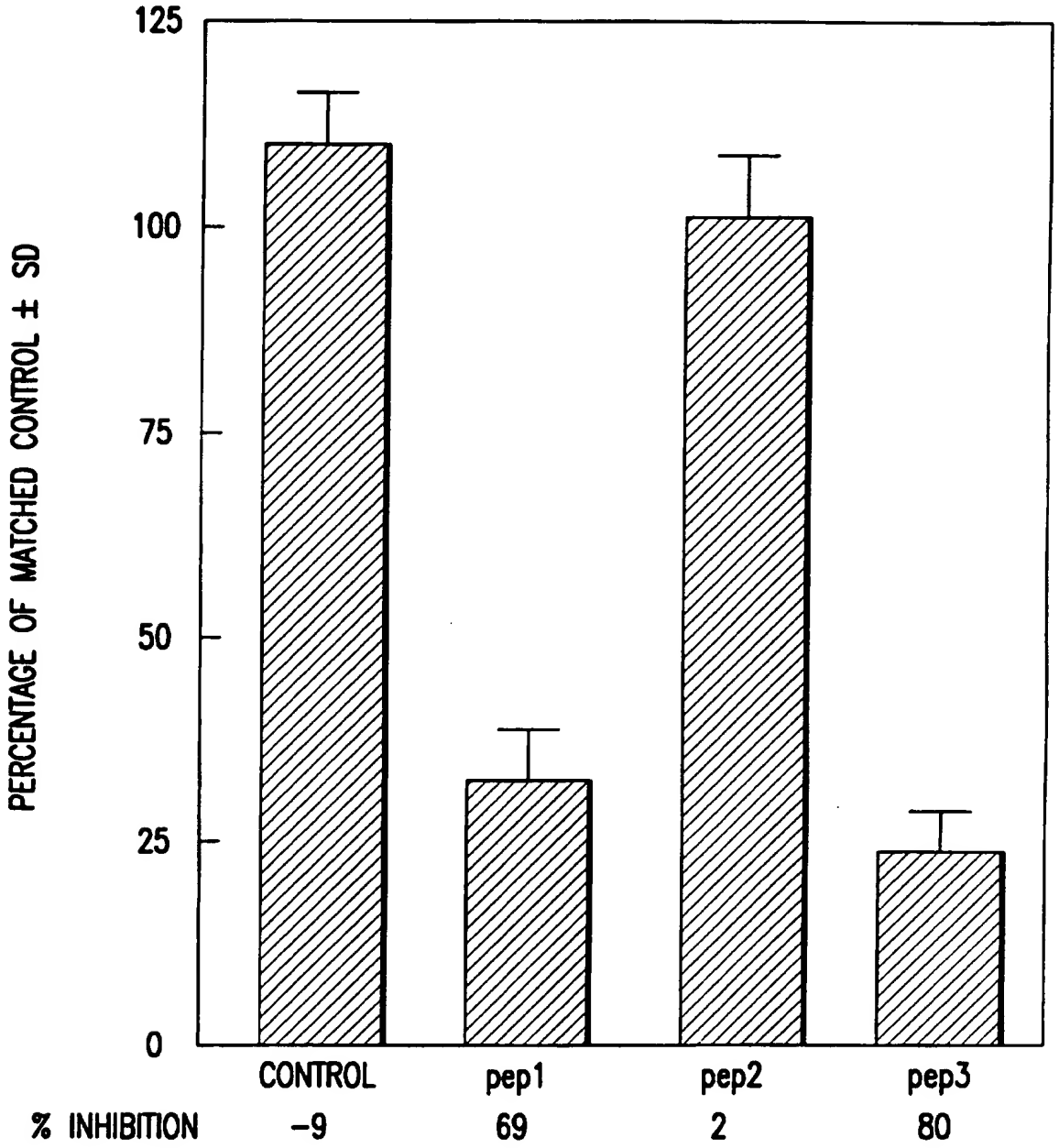
125
100
75
50
25
0
PERCENTAGE OF MATCHED CONTROL ± SD
CONTROL
pep1
pep2
pep3
% INHIBITION
-9
69
2
80

FIG.5

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 95/10166

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/705 A61K38/17 C12Q1/00 A61K39/395
C07K16/28 G01N33/53 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N A61K G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CELL, vol. 57, no. 7, 30 June 1989 NA US, pages 1123-1130, LISETTE LEYTON ET AL. '95 kd sperm proteins bind ZP3 and serve as Tyrosine kinase substrates in response to zona binding' cited in the application see the summary see page 1123, right column, paragraph 3 see page 1127, right column, paragraph 3 - page 1128, left column, paragraph 1 see page 1128, right column, paragraph 1 - paragraph 2 see page 1129, right column, paragraph 1 --- -/--	2

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

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Date of the actual completion of the international search	Date of mailing of the international search report
15 November 1995	14. 12. 95
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Montero Lopez, B

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 95/10166

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF REPRODUCTIVE IMMUNOLOGY, vol. 11, no. 3, July 1987 pages 157-166, H.D.M. MOORE ET AL. 'Monoclonal antibody agains a sperm antigen Mr 95000 inhibits attachment of human spermatozoa to the zona pellucida' cited in the application see abstract see page 160, paragraph 3 - page 162, paragraph 3 see page 163, paragraph 2 - page 165, paragraph 2 ---	1,17
A	WO,A,93 25233 (UNIVERSITY OF CONNECTICUT) 23 December 1993 see page 3, line 2 - line 17 see page 4, line 18 - page 5, line 12 see page 8, line 9 - line 16 see page 9, line 4 - line 26 ---	1-8, 11-16, 18-21
P,X	SCIENCE, vol. 269, no. 5220, 7 July 1995 LANCASTER, PA US, pages 83-86, D.J. BURKS ET AL. 'Interaction of a Tyrosine kinase from human sperm with the zona pellucida at fertilization'	2-8,17
P,Y	see abstract see page 84, left column, paragraph 2 - page 86, left column, paragraph 1; figures 2,5 ---	1,11-16, 18-27
P,Y	SCIENCE, vol. 269, no. 5220, 7 July 1995 LANCASTER, PA US, pages 39-40, R. JOHN AITKEN 'The complexities of conception' see page 39, left column, paragraph 2 see page 40, left column, paragraph 2 - right column, paragraph 1 -----	1,11-16, 18-27

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte nal Application No
PCT/US 95/10166

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9325233	23-12-93	AU-B-	4534893	04-01-94
		CA-A-	2137077	23-12-93
		EP-A-	0646015	05-04-95